2-12-2007

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Journal of Ecology, Vol. 95, No. 2 (February 12 2007) : 309-320. DOI.

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Summary

1. Multiple factors are known to influence tree seedling establishment, yet the degree to which these factors depend on each other and on spatial context is largely unknown. We examined the influence of herbaceous competition and water and nitrogen limitations on tree seedling establishment as functions

of distance from trees (within-site spatial context) and site history (between-site spatial context; as old-fields vs. savanna openings).

2. We grew *Quercus ellipsoidalis* E.J. Hill (pin oak) and *Q. macrocarpa* Michx. (bur oak) seedlings for 3 years in abandoned agricultural fields and savannas in central Minnesota, USA, near and distant from adult oak trees, with and without water and nitrogen resource additions, and with and without clipping of herbaceous vegetation (reducing above-ground competition).
3. The strongest treatment effects were found in response to distance from trees and clipping herbaceous vegetation. Ectomycorrhizal infection, year 1 foliar N concentrations, and survival were greater in seedlings growing near vs. distant from adult trees, while clipping herbaceous vegetation increased above-ground seedling biomass but reduced seedling heights, regardless of distance from adult trees.
4. There were conflicting effects of resource addition, which were dependent on clipping of herbaceous vegetation and site (savanna vs. old-field).
5. Distance from adult trees and clipping herbaceous vegetation appear to have largely independent effects. Thus, while being near trees benefits seedlings, probably via increased mycorrhizal infection, competition from herbaceous vegetation limits seedlings regardless of distance from trees. In contrast, the effects of resource addition were more context dependent, interacting significantly with herbaceous context and site.
6. The factors influencing seedling success can perhaps be best conceptualized as a series of largely independent environmental filters: seedlings near trees have increased mycorrhizal infection, nutrient uptake and survival, but face competition from herbaceous vegetation regardless of distance from trees. The slow encroachment of woody vegetation into old-fields and savanna openings in this region is likely to be the result of the net cumulative effect of such filters.

Introduction

Context-dependent effects are central to many theories in plant ecology, including facilitation (**Callaway *et al*. 2002**; **Maestre *et al*. 2005**) and competition (**Craine 2005**); yet, by necessity, many studies in ecology focus on only one or a few factors at a time. We studied oak seedling encroachment into grasslands as a system to understand the interactions and contexts of multiple factors influencing seedling success. Globally there has been increasing encroachment of woody plants into grasslands, causing changes in carbon storage (**Chapela *et al*. 2001**; **Jackson *et al*. 2002**), herbaceous plant communities (**Van Auken 2000**) and ecosystem function (**McCulley *et al*. 2004**). There are, however, some notable exceptions to the trend of woody encroachment. For example, abandoned agricultural fields (old-fields) and large savanna openings in parts of the mid-western USA appear to be in a state of arrested succession (**Fig. 1**), with tree establishment (mostly oaks) largely restricted to a narrow peripheral band (**Davis *et al*. 2005**).

Figure 1 Old-field 56, top, was abandoned from agriculture in 1958, yet oak regeneration is limited to a narrow band at the forest edge (visible at right edge of photograph). Large openings also occur in oak savannas, such as savanna site 106 (bottom).

Various explanations for the limited establishment of trees in old-fields have been proposed, including herbaceous competition, resource limitation, dispersal limitation, herbivory and a lack of mycorrhizal symbionts (e.g. **Inouye *et al*. 1994**; **Weltzin & McPherson 1999**; **Dickie *et al*. 2005**). Herbaceous competition is known to be an important limitation on seedling establishment, both in these sites (**Davis *et al*. 1999**; **Davis *et al*. 2005**) and in other tree-grass systems (**Hooper *et al*. 2005**; **Sharam *et al*. 2006**). Nitrogen is also an important limitation in these systems; however, the effects of increased nitrogen on oak seedlings can be neutral or negative due to increased competition with herbaceous vegetation (**Inouye *et al*. 1994**; **Davis *et al*. 1999**; **Lawson *et al*. 1999**). Mycorrhizal inoculum has also been shown to be low on seedlings distant from trees (**Dickie *et al*. 2005**), which might be expected to influence both resource limitation and competitive interactions of oak seedlings with herbaceous vegetation. A local-scale, lack of mycorrhizal infection distant from established trees has been implicated in the failure of regeneration in many other tree species (**Baylis 1980**; **Perry *et al*. 1989**; **Terwilliger & Pastor 1999**; **Nara & Hogetsu 2004**) and can be an important factor in succession (**Nara 2005**).

What is unclear from prior studies is whether, and how, the factors influencing seedlings interact with each other and with the spatial context in which they occur. We expected that the factors influencing seedling success would be strongly context dependent (as in **Davis *et al*. 1999**), particularly spatially within sites (whether seedlings were near or distant from established trees), and between sites with different histories (i.e. whether the opening was an old-field or a savanna opening). To test this, we manipulated three factors believed to be important in seedling establishment: herbaceous competition (by clipping), water availability (by watering) and nitrogen availability (by adding fertilizer) within two spatial-contexts: whether seedlings were near or distant from established trees (within-site spatial context) and whether the site was an old-field or savanna opening (between-site spatial context).

We expected that the factors limiting oak seedlings would vary with distance from adult trees because of the myriad ways that trees influence the abiotic and biotic environment (**Ehrenfeld *et al*. 2005**). For example, trees shade seedlings (**Weltzin & McPherson 1999**), compete below ground (**Coomes & Grubb 2000**), may increase (**Ko & Reich 1993**) or decrease soil moisture (**Anderson *et al*. 2001**) and can modify soil properties (**Weltzin & McPherson 1999**; **Reich *et al*. 2005**) and increase rates of nitrogen mineralization (**Reich *et al*.**

2001; **Dijkstra *et al*. 2006**). Trees also modify soil biota; seedlings growing within the root zone of adult trees may have increased infection by root pathogens (**Packer & Clay 2000**), but can also have greatly increased ectomycorrhizal infection, more diverse infection and can have increased growth compared with seedlings distant from trees (**Nara & Hogetsu 2004**; **Dickie *et al*. 2005**). Seedlings growing near adult trees may also be influenced by mycorrhizal networks, potentially reducing the cost to the seedling of establishing mycorrhizal infection (**Newman 1988**; **Simard & Durall 2004**). Nevertheless, distance from adult trees has not been widely integrated into prior studies of factors limiting oak regeneration. Understanding the effect of proximity to adult trees on seedling regeneration is critical, given that most oak regeneration occurs near trees at the edge of openings (**Inouye *et al*. 1994**), while manipulative studies of factors limiting tree establishment have generally taken place distant from trees. Site may also be an important factor because the herbaceous plant community of ecosystems differs between oak savannas and old-fields, and different communities are likely to have different effects on seedlings (**Davis *et al*. 2005**).

Methods

EXPERIMENTAL DESIGN AND TREATMENTS

We conducted the experiment at the Cedar Creek Natural History Area and Long-term Ecological Research site of the University of Minnesota, located in Anoka County, Minnesota, USA. We used a multilevel split-plot design (**Fig. 2**) to maximize our ability to detect significant interactions given inherent limitations on the availability of appropriate research sites and on our ability to apply certain treatments (such as backpack watering). We used six sites (**Table 1**), three in old-fields (Cedar Creek old-field numbers 56, 57 and 68) and three in oak savannas (Cedar Creek burn units 104, 106 and 115). Old-fields were likely to be oak savanna prior to conversion to agriculture; hence site differences reflect different histories. All sites had the same Typic Udipsamment type soils (**Grigal *et al*. 1974**). Old-fields were selected on the basis of having a relatively straight north edge running directly east–west (permitting us to equalize shading across distance treatments), no recent history of fire and little woody regeneration of any species. Savanna sites were selected on the basis of having sufficiently large openings devoid of trees to permit the establishment of treatments at least 10 m from any adult oak and having areas on the south side of trees where near-tree plots could be established with limited shading from adult trees. All savanna sites were maintained by periodic burning (**Peterson & Reich 2001**), with the most recent prescribed fire prior to the start of the experiment occurring in the spring of 2000 for all sites; site 104 also burned in a wildfire in the autumn of 2000. We prevented our plots from burning during the course of the experiment using watered fire-breaks and physical barriers. At least two of the savanna sites (104 and 115) had clear evidence of some former agricultural use (fencing wire, square edges, old farm implements).

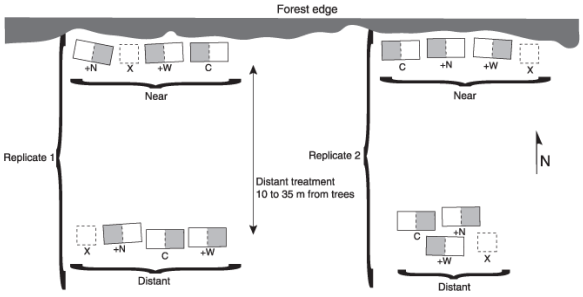

Figure 2 Schematic of experimental design in one of the six sites (three old-fields, three savannas), with plots near and distant from trees, nitrogen (+N), water (+W) and control (C) resource-addition treatment plots and uncaged, unclipped cage-control plots (X). Shaded portions of plots indicate clipped plots.

Table 1. Site descriptions for the three old-field and three savanna sites

Site*	Last year used for agriculture	Fires 1965–2002	Year of first fire†	Dominant herbaceous cover‡
Old-fields				
56	1958	0	NA	*Poa* spp. (32%), *Bromus* (29%), *Ambrosia* (7%)
57	1943	0	NA	*Poa* spp. (72%), *Ambrosia* (19%)
68	1957	0	NA	*Poa* spp. (65%), *Ambrosia* (12%), *Carex* (10%), *Toxicodendron radicans* (10%), *Physallis* (6%)
Savannas				
104	NA	31	1965	*Andropogon gerardi* (57%), *Carex* (12%), *Toxicodendron radicans*(10%), *Rhus* (9%), bare (6%), *Ambrosia* (6%)
106	NA	25	1966	*Carex* (23%), *Andropogon gerardi* (15%), *Ambrosia* (12%), *Lathyrus*(10%), bare ground (9%), *Schizacharium scoparium* (5%)
115	NA	4	1992	*Andropogon gerardi* (49%), bare (20%), *Carex* (11%), *Ambrosia*(10%)

* Site numbers are Cedar Natural History Area old-field numbers and burn-unit numbers.
† Years of first prescribed burn. Natural fire was probably common prior to 1900.
‡ Averages of mid-points of Daubenmire cover classes, for all herbaceous species comprising 5% or more of cover. Because these were taken as mid-points of cover classes, they do not necessarily sum to 100%.

Within each site we had two distance treatments (near-trees and distant), each of which was replicated twice. In old-fields, distant treatments averaged 20.3 m from trees (range 10.5–35 m), while in savannas distant treatments were an average of 14.4 m from trees (range 10.0–17.5 m). We located near-tree plots just beyond drip line, an average of 3.6 m from the base of adult trees in old-fields and 4.5 m from the base of adult trees in savannas. Focal trees were all *Quercus ellipsoidalis*, but *Q. macrocarpa* was present in all sites.

Within each distance-treatment replicate, we had three (1 m × 1.8 m) resource-addition plots (nitrogen added, +N; water added, +W; and control, C), each of which was further divided into two (1 m × 0.9 m) clipping treatments (clipped and unclipped). We applied 5 g m^{-2} y^{-1} N as granular NH_4NO_3 divided into three applications per year to the +N plots (*c*. 1 June, 1 July and 1 August of each year). We applied the equivalent of 1 cm precipitation two times per week to the +W plots with a backpack water tank through the growing season (June–August), except when > 1 cm of rain fell within 24 hours of a scheduled watering. Three times per year we clipped herbaceous vegetation on half of each plot, clipping as close to the ground level as possible with electric hand-held clippers. We removed vegetation clipped in spring of 2001 to avoid creating a heavy mulch layer and left subsequent clippings in place to minimize nutrient removal. We protected the plots with a 1.25-cm-mesh cage (1 m × 1.8 m) to reduce mammalian herbivory. To test for herbivore and cage effects we included an unclipped half-plot (1 m × 0.9 m) with no cage (designated X in **Fig. 2**).

We planted 15 pin oak (*Quercus ellipsoidalis*) and 15 bur oak (*Quercus macrocarpa*) seeds in a regular grid pattern, alternating species, in each half-plot (1 m × 0.9 m). We first planted in spring of 2001; however, because of low germination, we removed and discarded all seedlings in autumn of 2001 and replanted that autumn. We initiated clipping, +W and +N treatments in the spring of 2001 and continued until the final harvest in autumn 2004.

site variables

We measured soil moisture every 2–3 days over a period of 2 months (13 May 2002 to 12 July 2002), taking measurements at each near and distant treatment with a TDR (time domain reflectometry) probe (Trime-FM3 with 20 cm P3 rod probe; Ikmo, Ettlingen, Germany) adjacent to the C treatment plot and away from the +W plot. The objective of this sampling was to capture a strong drying trend and at least one re-wetting of the soil.

We characterized the herbaceous community in each of the unclipped plots by visually estimating percentage cover using Daubenmire cover classes, excluding species comprising less than 5% cover (Daubenmire class 1). We summarized the herbaceous cover data using principal components on herbaceous species found across three or more of the six sites. Statistics were performed on the mid-points of the cover classes. We used ion exchange resin to characterize available NO_3-N, NH_4-N, and PO_4-P following protocols described previously (**Hobbie 2005**). To protect against loss of samples, we buried four ion exchange resin bags at each distance replicate and used the average value from each set for analyses.

seedling response measurements

We counted seedlings periodically (three times in 2002, and twice in 2003 and 2004), marking all germinated seedlings with colour-coded, painted, galvanized nails placed in the soil adjacent to seedlings. Thus, we were able to track germination and survival independently and avoid any risk of counting a top-killed and resprouted seedling as a new germinant. In cases where both a seedling and the marker disappeared, we counted the seedling as killed; typically, this was associated with pocket gopher (*Geomys bursarius*) activity. During clipping of vegetation, a small number of seedlings were inadvertently cut. We recorded clipping independently of natural mortality and made a correction in the final analysis for calculation of survival.

We destructively harvested one seedling of each species from each treatment plot in the autumn of 2002 and again in the autumn of 2004. Growth was measured as height and above-ground biomass. We did not attempt to measure below-ground biomass, as the long taproots of seedlings and the dense herbaceous vegetation prevented complete root harvests. We measured foliar nitrogen concentrations in C and +W plots in 2002, and in C plots only in 2004, on an ECS 4010 element analyser (Costech Analytical, Valencia, CA, USA) at the University of Nebraska, compositing all leaves from a seedling into one sample. Financial constraints prevented measuring nutrient levels in all treatments.

We counted the percentage of root tips infected by ectomycorrhizal fungi in the C and +W treatment seedlings in 2002 and 2004. We did not identify mycorrhizal species; effects of distance on mycorrhizal infection were described previously (**Dickie & Reich 2005**). Preliminary measurements indicated that arbuscular mycorrhizal infection was rare, and were therefore discontinued.

STATISTICS

We tested the significance of all response variables by deletion from the maximal model of Site × Distance × Resource addition × Clipping shown in **Table 2**, with statistics run in R (version 2.0.1; **R Development Core Team 2005**). Binomial response variables (germination, survival) were tested using glmmPQL (generalized linear mixed models via penalized quasi-likelihood), while continuous response variables (e.g. height, mass) were tested using lme (linear mixed effect models). Models were simplified following the procedure described in **Crawley (2002**), deleting least-significant terms until a minimal adequate model was obtained. Second-order and third-order interactions were considered significant at $\alpha = 0.01$; main effects and first-order interactions were considered significant at $\alpha = 0.05$ following **Crawley (2002**). For analysis of the effect of the cage, we compared results in the unclipped C plot with the cage-control plots.

Table 2. Summary of *P*-values for all main effects and interactions. *P*-values are for minimal adequate models for all response variables showing significant responses to at least one factor at $P < 0.05$ for main effects and first-order interactions and $P < 0.01$ for higher-level interactions (Crawley 2002), as found by deletion from the maximal model: response ~ Site × Distance × Resource addition × Clipping (following Crawley 2002). Terms included in the minimal adequate model as marginal-to-significant interaction are indicated by *, factors not included in the minimal adequate model are indicated by '.'. Interactions that never had significant effects (S × D × R × C, S × D × C, and S × R × C) are omitted, as are response variables that showed no significant responses (bur germination, bur oak mass at year 1). Myc = mycorrhizal infection

Response	Site (S)	Distance (D)	Resource addition (R)	Clipping (C)	S × D	S × R	S × C	D × R	D × C	R × C	S × D × R	**D × R × C**
Pin germination	.	.	*	*	.	.	.	.	.	0.0045	.	.
Pin survival	*	0.0060	*	*	.	.	0.019	.	.	0.0064	.	.
Bur survival	*	*	.	.	0.049	.	.	.	.	.	.	.
Pin Myc year 1	.	< 0.0001	.	.	.	.	.	.	.	.	.	.
Bur Myc year 1	.	*	*	*	.	.	.	*	*	*	.	0.0063
Pin Myc year 3	.	0.0001	.	0.027	.	.	.	.	.	.	.	.
Bur Myc year 3	.	0.0001	.	.	.	.	.	.	.	.	.	.
Pin N year 1†	.	0.0036	.†	.	.	.	.	.	.	.	.	.
Bur N year 1†	.	0.0016	.†	.	.	.	.	.	.	.	.	.
Pin N year 3†	0.047	0.021	–	.	.	–	.	–	.	–	–	–
Bur N year 3†	0.047	.	–	.	.	–	.	–	.	–	–	–
Pin height year 1	.	.	.	< 0.0001	.	.	.	.	.	.	.	.
Bur height year 1	.	.	.	0.0008	.	.	.	.	.	.	.	.
Pin height year 3	*	*	*	0.0004	*	*	.	*	.	.	0.0068	.
Bur height year 3	.	*	0.028	*	.	.	.	.	0.043	.	.	.

Pin mass year 1	*	.	*	.	.	0.023	.	.	.	.	.	.
Pin mass year 3	.	.	.	0.0028	.	.	.	.	.	.	.	.
Bur mass year 3	.	.	.	0.0007	.	.	.	.	.	.	.	.

† Foliar N contents measured only in the control and +W resource-addition treatments in year 1, and only in control treatments in year 3.

All hypotheses were tested across full factorial design for interactions. Unless specifically mentioned, reported effects were significant across (and did not interact with) other experimental treatments.

Results

Distance from trees (**Fig. 3**) and clipping of herbaceous vegetation (**Fig. 4**) both had many significant main effects, and relatively few interactions with other treatments (**Table 2**). Distance primarily influenced survival, mycorrhizal infection and foliar nutrient concentrations, while clipping influenced germination and survival of pin oaks, and the growth of both oak species. Resource addition (**Fig. 5**) had fewer significant effects, most of which were marginal-to-significant interactions, with more-significant effects for pin oaks (significant effects on germination, survival, above-ground mass in year 1 and heights in year 3) than for bur oaks (significant effects only on mycorrhizal infection in year 1 and heights in year 3).

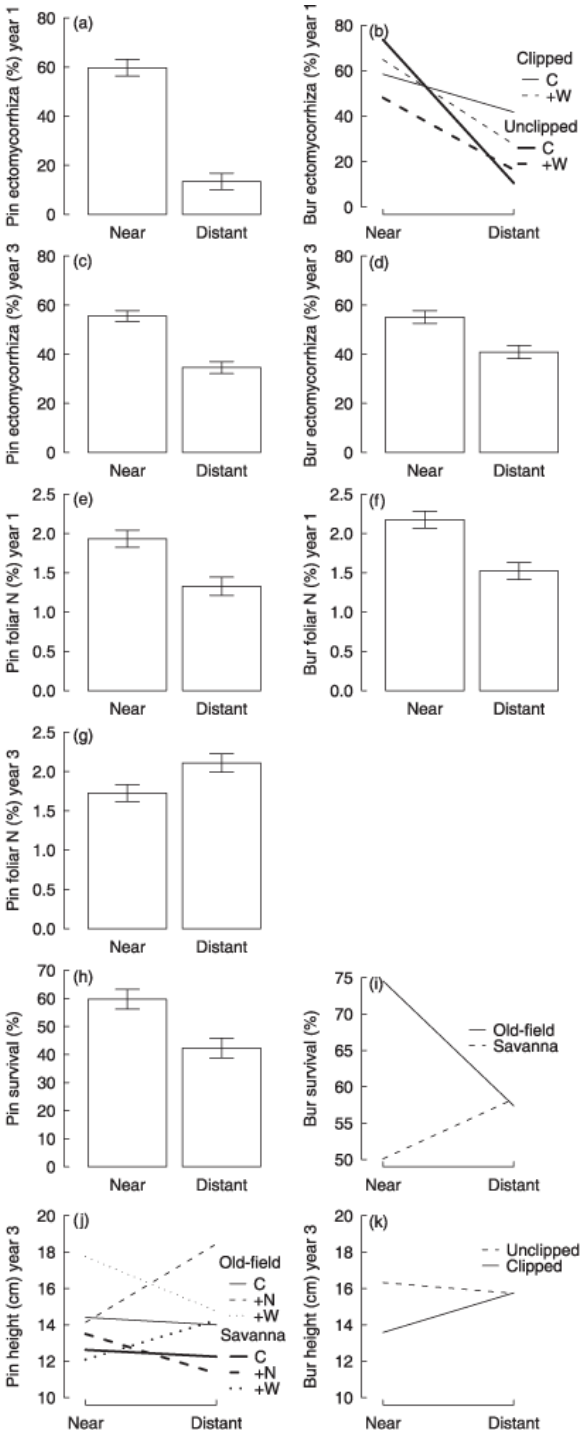

Figure 3 Significant effects of distance from trees on mycorrhizal infection (a–d), foliar N concentrations (e–g), survival to year 3 (h, i) and height (j, k) of pin and bur oak seedlings. Error bars are 1 SE. In b and j, C = resource addition control, +W = water addition treatment and +N (j only) = nitrogen addition treatment. All effects shown significant at $P < 0.05$, except significant three-way interactions, which were significant at $P < 0.01$; see **Table 2** for exact *P*-values.

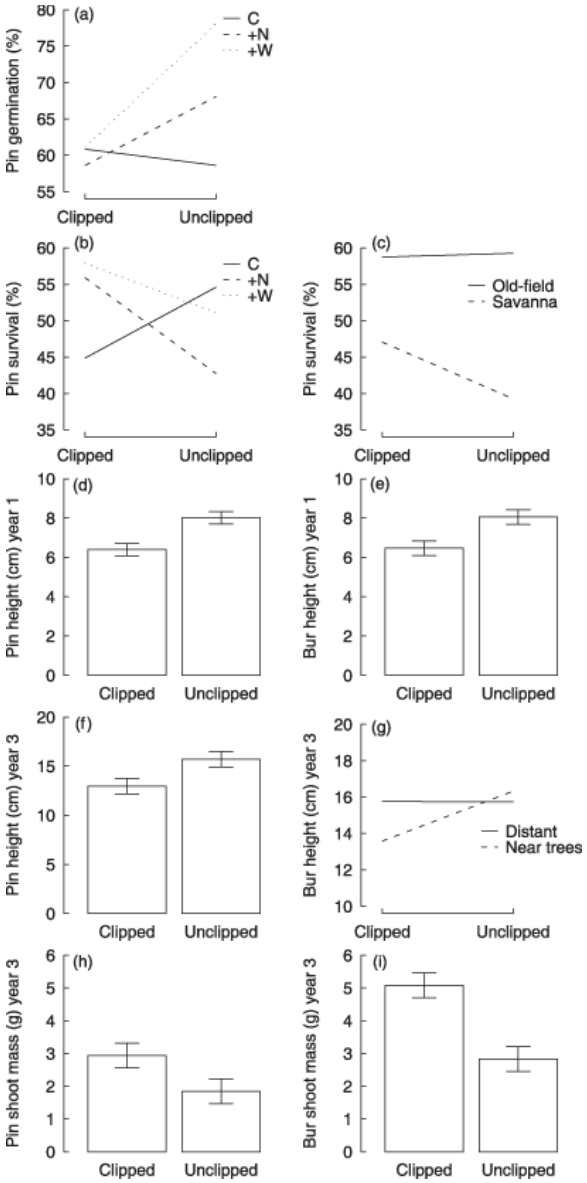

Figure 4 Significant effects of clipping on germination (a), survival to year 3 (b, c), height (d–g), and mass (h, i) of pin and bur oak seedlings. In a and b, C = resource addition control, +W = water addition treatment and +N = nitrogen addition treatment. All effects shown significant at $P < 0.05$; see **Table 2** for exact *P*-values.

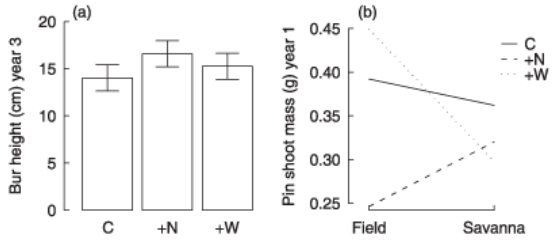

Figure 5 Significant effects of resource addition on year 3 bur oak heights (a) and year 1 pin oak shoot mass (b). Significant interactions of resource addition with distance are shown in **Figure 3**(b, j); and significant interactions with clipping are shown in **Figure 4**(a, b). Treatment codes are C = resource addition control, +W = water addition treatment and +N = nitrogen addition treatment. All effects shown significant at $P < 0.05$; see **Table 2** for exact *P*-values.

distance from trees

Mycorrhizal infection was higher near trees for both pin and bur oak seedlings at both year 1 and year 3 (Fig. 3a–d; pin oaks year 1, $F = 154.12$, $P_{1,11} < 0.0001$; pin oaks year 3, $F = 41.70$, $P_{1,10} = 0.0001$). Mycorrhizal infection of bur oak in year 1 showed a significant interaction between distance, clipping and resource addition (Fig. 3b; $F = 8.48$; $P_{1,34} = 0.0063$), while mycorrhizal infection of bur oak at year 3 had a significant main effect of distance (Fig. 3d; $F = 32.59$, $P_{1,11} = 0.0001$).

Foliar N concentrations were higher in seedlings near trees than distant from trees in year 1 for both pin oak seedlings (Fig. 3e; $F = 14.25$, $P_{1,10} = 0.0036$) and bur oak seedlings (Fig. 3f; $F = 18.52$, $P_{1,10} = 0.0016$). At year 3 the pattern had reversed for pin oaks (Fig. 3g, $F = 7.86$; $P_{1,9} = 0.021$), while foliar N of bur oak at year 3 was not significantly affected by distance.

Survival was higher near trees than distant from trees for pin oaks in both sites (Fig. 3h; $F = 11.49$, $P_{1,11} = 0.0060$) and for bur oaks only in old-fields (Fig. 3i; Site × Distance interaction, $F = 5.02$; $P_{1,10} = 0.049$). There was no effect of distance from trees on growth of either oak species at year 1. Bur oak height at year 3 was lower in near-trees in unclipped plots, but showed no response to distance in clipped plots (Fig. 3k; $F = 4.31$, $P_{1,51} = 0.043$). Distance from trees influenced how heights of pin oaks at year 3 responded to resource addition and site, but had no clear direct effect (Fig. 3j; significant three-way interaction, $F = 4.88$, $P_{3,31} = 0.0068$). In control plots there was no effect of distance on pin oak seedling heights; watering increased height growth in near-tree plots in old-fields, and in distant plots in savannas; while N additions increased heights of pin oaks at year 3 in distant plots in old-fields and to a lesser degree in near-tree plots in savannas, but had a small negative effect on heights in distant plots in savannas.

clipping of herbaceous competition

Germination of pin oaks was greater in unclipped plots with water and unclipped plots with nitrogen additions than in clipped plots or unclipped control plots (Fig. 4a; $F = 5.6$; $P_{2,69} = 0.0045$). Germination of bur oaks was also marginally higher in unclipped than clipped plots, but this relationship was not significant ($P = 0.058$; data not shown).

Survival of pin oaks was significantly higher in clipped +W and +N plots than controls, and lower in unclipped +N plots than in unclipped +W or control plots (Fig. 4b; $F = 5.46$, $P_{2,66} = 0.0064$). Survival was also higher in clipped savanna than unclipped savanna plots (Fig. 4c; $F = 0.019$, $P_{1,66} = 0.019$), but there was no difference in survival in old-fields.

In year 1, clipping reduced heights of both pin (Fig. 4d; $F = 19.59$, $P_{1,64} < 0.0001$) and bur (Fig. 4e; $F = 12.51$; $P_{1,65} = 0.0008$) oak seedlings. In year 3, clipping reduced heights of pin oak (Fig. 4f; $F = 14.45$; $P_{1,50} = 0.0004$), and bur oak in near-tree plots but not distant plots (Fig. 4g; $F = 4.31$, $P_{1,51} = 0.043$). In contrast to the effects of clipping on height, in year 3 clipping increased above-ground biomass of both pin (Fig. 4h; $F = 9.59$, $P_{1,71} = 0.0028$) and bur oak seedlings (Fig. 4i; $F = 12.53$, $P_{1,71} = 0.0007$). Thus, seedlings were shorter but had more above-ground biomass in clipped plots than in unclipped plots; seedlings in unclipped plots were often noticeably etiolated.

Ectomycorrhizal infection of pin oaks at year 3 was higher in clipped than unclipped plots (49.4 ± 2.9% and 43.6 ± 2.9% of root tips, respectively; $F = 5.14$, $P_{1,53} = 0.027$), while ectomycorrhizal infection of bur oaks at year 1 was also higher in clipped plots than unclipped plots, except in near-tree C plots, where the extent of infection was similar in clipped and unclipped plots (Fig. 3b; $F = 8.48$; $P_{1,34} = 0.0063$).

resource addition (water and nitrogen)

The effects of resource addition were largely as part of significant interactions (**Table 2**), with the sole exception of heights of bur oaks at year 3, which were higher in +N plots than controls, with +W plots intermediate (**Fig. 5a**; $F = 3.93$, $P_{2,40} = 0.0276$). The +W treatment and to a lesser extent the +N treatment resulted in increased germination of pin oaks in unclipped but not clipped plots (**Fig. 4a**; $F = 5.6$; $P_{2,69} = 0.0045$). In clipped plots, both +W and +N increased survival of pin oaks, while in unclipped plots survival was higher in control and +W plots than in +N plots (**Fig. 4b**; $F = 5.46$, $P_{2,66} = 0.0064$).

Nitrogen addition strongly reduced above-ground biomass of pin oaks at year 1 in old-fields, with a smaller negative effect in savannas, while water addition caused a small increase in above-ground biomass of pin oak at year 1 in old-fields and a small decrease in above-ground biomass of pin oak at year 1 in savannas (**Fig. 5b**; $F = 4.14$, $P_{2,44} = 0.023$). Heights of pin oaks at year 3 showed a significant three-way interaction of site, distance and resource addition (**Fig. 3j**; $F = 4.88$, $P_{3,31} = 0.0068$); watering increased heights at year 3 in near-tree plots in old-fields, and in distant-from-tree plots in savannas, while N additions increased heights of pin oaks at year 3 distant-from-tree plots in old-fields and to a lesser degree in near-tree plots in savannas, but had a small negative effect on height, distant from trees in savannas.

site (old-fields vs. savannas)

The only significant main effect of site was on foliar N concentrations at year 3, which were lower in savannas than in old-fields for both pin oaks and bur oaks (pin oaks: old-fields, 2.07 ± 0.11%; savannas, 1.67 ± 0.10%; $F = 8.03$, $P1,_4 = 0.047$; and bur oaks: old-fields, 2.01 ± 0.11%; savannas, 1.57 ± 0.11%; $F = 8.07$, $P_{1,4} = 0.047$). There were also significant interactions of site with distance, clipping and resource addition (**Table 2**), described above and in **Figs 3(i,j), 4(c) and 5(b)**.

site variables

Maximum soil moisture TDR measurements were slightly higher near trees than distant from trees (21.0 ± 0.8% near trees vs. 20.2 ± 0.8% distant; $F = 5.25$, $P_{1,11} = 0.043$). However, mean and minimum soil moisture did not vary between site types or distances, and there were no obvious differences in drying and wetting trends. There were no significant differences between sites or distances in ion-exchange-resin-available NO_3-N, NH_4-N and PO_4-P measurements.

Herbaceous vegetation was influenced by site habitat type, and in savannas, by watering. Principal component axes one and two explained 50.0% and 30.0% of the variance, respectively. There were no significant effects of site or resource addition on PCA axis 1, but there was a significant site effect and interaction of site with resource-addition on axis 2 ($F = 47.86$, $P_{1,4} = 0.0023$ and $F = 3.4$, $P_{3,66} = 0.023$, respectively).

cage effects

The only significant effects of cages were on germination of pin oaks and height growth of bur oak (at both year 1 and 3). Germination of pin oaks was higher in uncaged controls than caged controls (74 ± 3% and 59 ± 5%, respectively, $P_{1,23} = 0.047$; not shown). There was a significant interaction between cages and distance for heights of bur oak at year 1 and year 3, with a negative effect of cages on heights at year 1 distant from trees, but a positive effect on heights at year 1 near trees (Distance × Cage interaction; $F = 9.70$, $P_{1,20} = 0.0055$; data not shown), and a positive effect of cages on heights of bur oak at year 3 ($F = 6.99$, $P_{1,18} = 0.017$; data not shown).

Discussion

The factors limiting oak seedling establishment can be conceptualized as a series of largely independent environmental filters, the combination of which determines if tree seedlings establish and survive. Both herbaceous competition and distance from trees influenced seedling survival and growth, yet there was little

evidence of interactions between them. Thus, while being near trees benefited seedlings, probably via increased mycorrhizal infection, competition from herbaceous vegetation remains an important limitation on seedlings regardless of distance from trees. The effects of resource addition, in contrast, appeared to be largely dependent on herbaceous context and on site. Adding resources had strong indirect effects by increasing competition from herbaceous vegetation (**Davis *et al*. 1999**; **Turkington *et al*. 2002**). Overall, the effects of resource addition were less important than distance from trees or clipping herbaceous-competition in influencing seedling success.

distance from trees

The increased mycorrhizal infection and foliar N concentrations of seedlings near adult trees was largely consistent with prior studies of oaks (**Dickie *et al*. 2002**; **Dickie *et al*. 2005**) and other species (**Baylis 1980**; **Perry *et al*. 1989**; **Terwilliger & Pastor 1999**; **Simard & Durall 2004**; **Nara 2005**), although the present study was the first time increased survival of oak seedlings near established trees has been observed. This contrasts with the expectation that survival will be lower in seedlings near conspecific trees due to disease (**Connell 1970**; **Janzen 1970**; **Packer & Clay 2000**; **Wills *et al*. 2006**). The difference between savannas and old-fields in the effect of proximity to trees on bur oak survival is particularly interesting: facilitation is generally expected to be more important in harsh sites (**Callaway *et al*. 2002**; **Lortie & Callaway 2006**), yet for bur oaks facilitation was found in old-fields (which had higher overall survival) and not in savannas. This finding supports suggestions that there may be limitations to the hypothesis that facilitation is more important in stressful sites (**Maestre *et al*. 2005**; **Riginos *et al*. 2005**; **Maestre *et al*. 2006**).

The positive effects of being near established trees were probably due to increased mycorrhizal infection (**Dickie & Reich 2005**), which would explain increased foliar N concentrations in seedlings in year 1. Other studies have found higher N availability near trees than distant from trees (**Weltzin & McPherson 1999**; **Reich *et al*. 2001**; **Dijkstra *et al*. 2006**), which could also explain higher seedling foliar N near trees; however, using ion-exchange-resin measurements, we did not detect a significant effect of distance on available N, nor did bio-indicators of available N show a distance effect in earlier studies at this site (**Dickie *et al*. 2005**). Our near-tree plots were just outside drip-line, while previous measures of soil N near trees were taken under canopy. Assuming that our measurements at the actual locations of the plots are the best indication of available N, increased mycorrhizal infection is the most likely explanation for elevated foliar N concentrations in seedlings near adult trees.

In other studies, low mycorrhizal infection distant from established ectomycorrhizal vegetation has been linked to reduced growth of seedlings (**Baylis 1980**; **Perry *et al*. 1989**; **Dickie *et al*. 2002**; **Simard & Durall 2004**; **Dickie *et al*. 2005**); this phenomenon was not observed in the present study. Pin oak foliar N concentration also showed a reversal of pattern by year 3, with higher N distant from trees than near trees, which is inconsistent with prior results (**Dickie *et al*. 2005**). These contrasting results may reflect the complex interactions of trees with seedlings: while trees may facilitate seedling mycorrhizal infection they are also competing with seedlings for light, soil nutrients and water. The net outcome of these interactions may depend on the distance between trees and seedlings and available resource levels (**Dickie *et al*. 2005**).

clipping of herbaceous competition

There were positive effects of clipping on seed- ling survival and growth, with increased biomass but reduced heights in clipped plots for both pin oak and bur oak. We had expected that the effect of clipping would be greater in old-fields than in savannas based on the suggestion by **Davis *et al*. (2005**) that non-indigenous grasses in old-fields inhibit oak seedling growth more than the native herbaceous vegetation of savannas. This was not found to be true. It should be noted, however, that our treatment (above-ground clipping) probably reduced,

but may not have entirely removed, below-ground influences, which Davis and colleagues suggest are the primary mechanism of oak growth inhibition by non-native grasses (**Davis *et al*. 2005**).

Higher germination of pin oaks in unclipped +W and +N plots than in clipped plots was likely related to soil moisture. In unclipped plots herbaceous vegetation shaded the ground, creating better conditions for germination and protecting young seedlings from desiccation. This pattern was not observed for bur oaks, perhaps because bur oak germinates in the autumn, when moisture is typically less limiting. Adding resources may have benefited herbaceous vegetation more than oak seedlings (**Davis *et al*. 1999**); increased herbaceous growth would explain increased germination in +N and +W plots (via increased shading creating cooler, moister conditions in surface soils).

Clipping also increased mycorrhizal infection of pin oak at year 3 and of bur oak at year 1 (except in +W near-tree plots). This is consistent with observations of increased mycorrhizal infection of oak seedlings following partial canopy removals (**Zhou & Sharik 1997**) and may reflect competition for light reducing the availability of photosynthate to support the costs of supporting mycorrhizal fungi (**Haskins & Gehring 2005**; **Corrêa *et al*. 2006**).

resource (water and nitrogen) addition

Resource addition had few direct effects (only an increase in heights of bur oak at year 3 in the +N treatment), but had a number of interactions with other treatments. These complex interactions may reflect the opposing direct effects and indirect effects of resource addition: adding water and nitrogen influenced both pin oak seedlings and herbaceous competition. Grasses can compete strongly with trees for nitrogen (**Clinton & Mead 1994**) and in some cases the indirect effect of adding resources via increased competition with herbaceous vegetation may result in a net negative effect on oak seedlings (**Davis *et al*. 1999**; **Turkington *et al*. 2002**). This could be considered as evidence for weak context dependency; the local competitive context of seedlings determines their response to nutrients. Most of the effects of resource addition were apparent only on pin oak seedlings, with few effects of resource addition on bur oak seedlings. Bur oaks germinate in the autumn and quickly establish a deep taproot. Thus, bur oaks may be less responsive to resource addition (both direct and indirect effects).

site effects

Where significant site effects were found, seedlings were generally favoured in old-fields as compared with savannas. Both pin and bur oak survival were higher in old-fields, pin oak shoot mass was generally higher at both year 1 and 3, and year 3 foliar N was higher in old-fields.

Our selection of large openings in savannas may have biased our sample towards particularly harsh sites: the absence of oak regeneration in these sites may reflect poor conditions for oak establishment. At least two of our savanna sites also had some evidence of past agricultural use and it is likely that the third site may also have been grazed or otherwise influenced in the past (although probably much longer ago than in the old-fields). Nonetheless, the vegetation in all three savanna sites was distinct from old-fields, with greater dominance by native species and fewer non-indigenous grasses. Site differences in herbaceous communities (apparent as significant Site × Distance interaction on PCA axis 2) were mainly driven by a higher abundance of *Poa* in old-fields and *Andropogon* and *Carex* in savannas. The significant interaction of site and resource-addition treatment was the result of a reduction in the abundance of *Helianthus* in watered savanna plots, while *Helianthus* was largely absent from old-fields regardless of watering.

Conclusions

Proximity to established trees and competition from herbaceous vegetation both had strong effects on seedling survival and growth, but with little evidence of interaction with each other. This implies that while being close to established trees is an important determinant of increased seedling mycorrhizal infection and nutrient uptake, seedling success remains strongly limited by herbaceous competition both near and distant from trees. This confirms earlier studies on the effects of distance (**Dickie *et al*. 2005**) and the effects of herbaceous competition (**Davis *et al*. 1999**), and suggests that each process can be understood in the absence of the other.

One way to conceptualize the limits on oak recruitment is as a series of independent environmental filters. Relatively few acorns disperse distant from trees (**Weltzin & McPherson 1999**). Our results suggest that these few are at a significant disadvantage in terms of mycorrhizal infection, nutrient uptake and survival. In addition, all oak seedlings, regardless of distance from trees, must successfully compete with herbaceous vegetation. The slow woody encroachment of oaks into old-fields and savannas is likely to be the result of the combined effects of these environmental filters.

Acknowledgements

A large number of research interns contributed to this experiment; we specifically thank A. Gunderson, M. Goldstein and R. Guza, who took leadership roles as interns on this project. J. Trost, S. Barrott and C. Buschena provided key technical support. Vital help with statistical analysis was given by G. Forrester and S. Richardson. R. Allen, M. Davis, D. Peltzer, F. Gilliam and two reviewers provided helpful comments. The US National Science Foundation LTER program funded this research (NSF/DEB 0080382). I.A.D. was also supported by funds from the Foundation for Research, Science and Technology (FRST, NSOF) of New Zealand during the analysis and writing stage of this research.

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